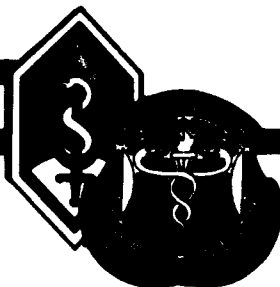


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USAARL Report No. 93-30



**Effect of Photoperiod
on Blood Cholinesterase Activity
and Melatonin Concentrations in Adult Cats**

By

**Alfred T. Townsend
Albert W. Kirby
Carolyn D. Pope
George Evans**

Sensory Research Division

August 1993

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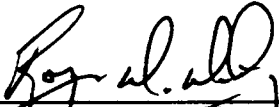
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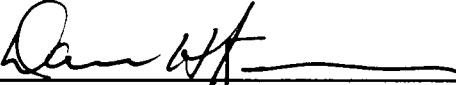
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<p>We studied the effects of photoperiod on plasma melatonin (MEL) concentrations, butyryl-cholinesterase (BUChE), and blood acetylcholinesterase (AChE) activity in 7 adult cats. Cats were exposed to a 14:10 light-dark (LD) cycle with lights on from 0630 to 2030 for 3 months before blood sampling. Blood samples were obtained every 4 hours for up to 52 hours by repeated venipuncture of the jugular vein in three animals, while four animals had indwelling catheters implanted prior to sampling. All animals were handled repeatedly for several weeks prior to the study to minimize stress. MEL concentrations were measured in plasma by radio-immunoassay. Plasma BUChE activity and whole blood AChE activity were determined from the same blood samples. In all of the animals, mean MEL concentrations were higher during the dark period of the LD cycle, while plasma BUChE and blood AChE activity had no observable change in response to the LD cycle. These findings indicate that MEL secretion, but not</p> <p>(continued)</p>					
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BUChE and AChE activity in cats, is responsive to changes in photoperiod. Under controlled conditions, secretion of MEL may be used as a control marker for physiological rhythmicity in cats when studying the effects of photoperiod on other compounds.

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Introduction

Activity of synaptically released acetylcholine (ACh) is terminated by the degradative enzyme acetylcholinesterase (AChE), or true cholinesterase. There is a second class of cholinesterase found in living systems, pseudocholinesterase (butyrylcholinesterase, BUCHE) which preferentially hydrolyzes higher choline esters. Some of our previous studies with cholinesterase inhibitors relate decrements in the visual evoked response (VER) to inhibition of AChE or BUCHE (Harding, Wiley, and Kirby, 1983; Harding, Kirby and Wiley, 1985; Kirby, Harding and Wiley, 1987). Initial decrements in the VER appear to be related to blood activity of cholinesterases. In addition to AChE inhibition, other neurochemical alterations have been reported after exposure of animals to organophosphate (OP) (Kirby, Harding, and Wiley, 1987).

It is essential to the interpretation of results from studies involving cholinesterase inhibitors to know if a circadian variation in cholinesterase activity is present, and if so, how much of the observed results can be explained on the basis of that rhythm. Establishing baseline enzyme activity at a peak or base of a circadian period could result in a significant difference in the calculated percent inhibition.

Circadian variations in AChE have been described in many species, including the mouse brain (Lewandowski, 1983), the rat brain (Schiebeler and Mayerbach, 1974), the cockroach nervous system (Vijayalakshmi, Mohan, and Babu, 1977), the central nervous system of the grasshopper (Vijayalakshmi and Babu, 1978), and it has been suggested in human brain enzymes (Perry, et al., 1977). Experimental conditions and amount of demonstrated rhythmicity vary widely between species and laboratories. We know of no previous studies investigating circadian variations in AChE and BUCHE in the cat.

To lend validity to changes in BUCHE and AChE activities which might be linked to the LD cycle, plasma levels of melatonin (MEL) also were determined. Leyva et al. (1984) showed that MEL concentration in cat blood is responsive to the dark phase of the LD cycle. Melatonin plasma levels were shown to increase in the dark period due to an increase in the activation of the enzyme N-acetyltransferase (Rudeen, Reiter, and Vaughan, 1975; Reiter, 1987). In this study, we report the effects of the LD cycle on AChE and BUCHE activity and melatonin secretion in cats.

Materials and methods

Five adult male and two noncycling female cats (mixed breed, 3.1-4.9 Kg) were used in this study and were maintained in rooms

at 21°C under a 14:10 LD cycle (600 lux) for approximately 3 months before the experiment. Animals were free of disease and received food (Purina high protein fortified chow) and water ad libitum. Blood samples were obtained every 4 hr for 52 hours by repeated venipuncture of the jugular vein in 3 animals, while 4 animals had indwelling catheters surgically implanted in the jugular vein prior to sampling. All animals were monitored by hematocrit to ascertain if the amount of blood withdrawn altered total blood volume. In an effort to minimize stress during sample collection, animals were placed in a canvas bag several times a day for several weeks prior to the study.

AChE and BUCHE activity were determined according to the method of Ellman et al. (1961). AChE activity is expressed as moles of acetylthiocholine iodide hydrolyzed/min/rbc $\times 10^{10}$. BUCHE activity is expressed as moles of butyrylthiocholine iodide hydrolyzed/ μ l plasma/min.

All immunoreagents and procedures employed for the determination of MEL in this study were supplied by Stockgrand LTD, Department of Biochemistry, University of Surrey, Guildford Surrey, UK. Briefly, 500 μ l of sample or standard was added to the assay tubes in duplicates. Two hundred μ l of antiserum (G/S/704-8483) were added and the tubes were vortexed and incubated at room temperature for 30 minutes. One hundred μ l of 3 H-melatonin then were added, vortexed and incubated at 4°C for 18 hours in a cold room. The antibody-bound melatonin was separated from the free fraction by vortexing and subsequent incubation with dextran coated charcoal for 15 minutes at 4°C. The resulting mixture was centrifuged at 1500 x g at 4°C for 15 minutes. An aliquot of the supernatant was removed and placed into vials containing 6 ml of scintillant. The vials then were shaken at room temperature for 1 hour and counted by liquid scintillation spectrometry. Repeated analysis of this RIA over a 3-month period revealed a limit of detection of 2.2-5 pg/tube. Parallelism existed between standard curves prepared using pooled stripped human plasma and pooled stripped feline plasma. Intra and interassay coefficients of variation were 7.6 and 4.1 percent, respectively. Specificity was demonstrated through the use of a high performance liquid chromatography (HPLC) method utilizing electrochemical detection (Medford and Barchas, 1980). Concentrations of MEL (1-3 ng) were injected through the HPLC system, and peaks were identified by retention. MEL was quantified by peak height.

Differences between light and dark concentrations of MEL or BUCHE and AChE activity values in the blood of individual animals were assessed using one-way analyses of variance and covariance with repeated measures. The minimum acceptable level of significance in all cases was $p < 0.05$.

Results

Figure 1 illustrates the effect of the LD cycle on blood AChE activity in male and female cats. When comparing animal gender, AChE activity did not differ significantly; therefore, results from male and female cats were combined. AChE activity did not show any significant elevation or decrement in response to photoperiod. These findings demonstrate that blood AChE activity in cats does not change significantly during a 14:10 LD cycle.

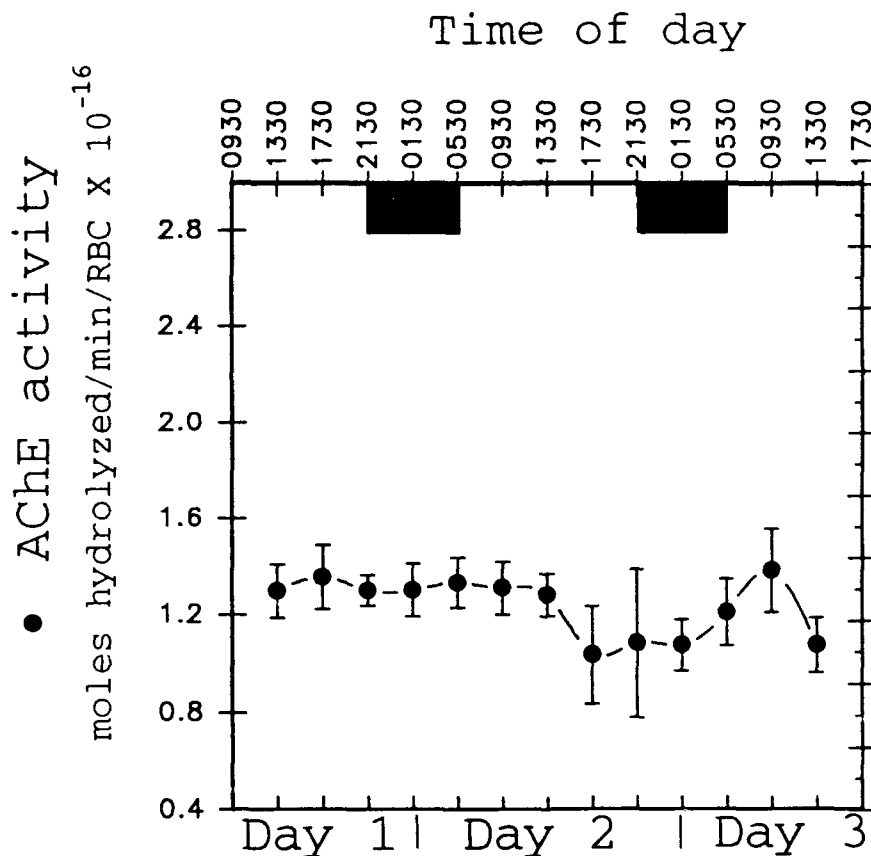


Figure 1. The effect of photoperiod on blood acetylcholinesterase activity in cats determined at 4-hour intervals. Data are represented as the mean \pm SEM of seven animals assayed in duplicate at each time period.

Figure 2 illustrates the effect of the LD cycle on plasma BUCHE activity in male and female cats. As with AChE activity, BUCHE activity did not differ between animals of different sex. In addition, BUCHE activity did not show any significant elevation or decrement in response to the LD cycle. These findings demonstrate that plasma BUCHE activity in cats does not change significantly during a 14:10 LD cycle.

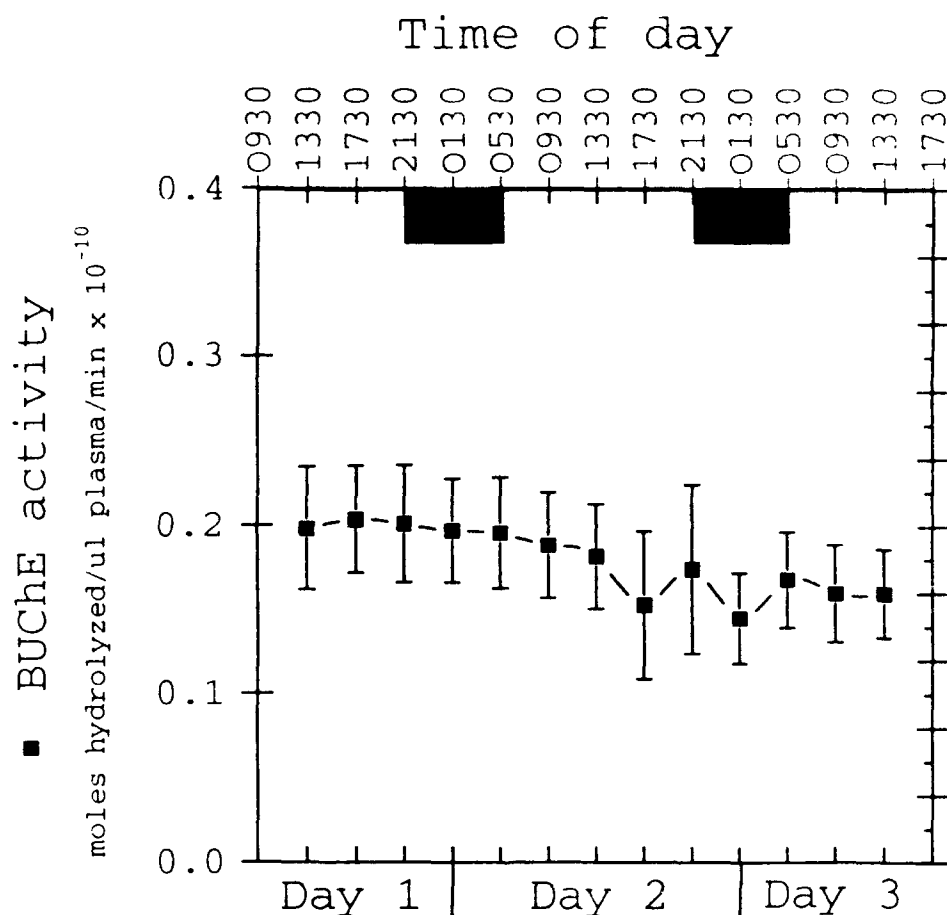


Figure 2. The effect of photoperiod on plasma butyrylcholinesterase activity in cats determined at 4-hour intervals. Data are represented as the mean \pm SEM of seven animals assayed in duplicate at each time period.

Figure 3 illustrates the effect of the LD cycle on plasma MEL concentrations in adult cats. In all of the animals studied, mean MEL concentrations were higher during the dark period of the photoperiod regime. This pattern and magnitude of change appears to be consistent in animals regardless of their gender. In agreement with an earlier report (Leyva, Addiego, and Stabenfeldt, 1984) these findings indicate that MEL concentrations are sensitive to the LD cycle and are elevated in the dark.

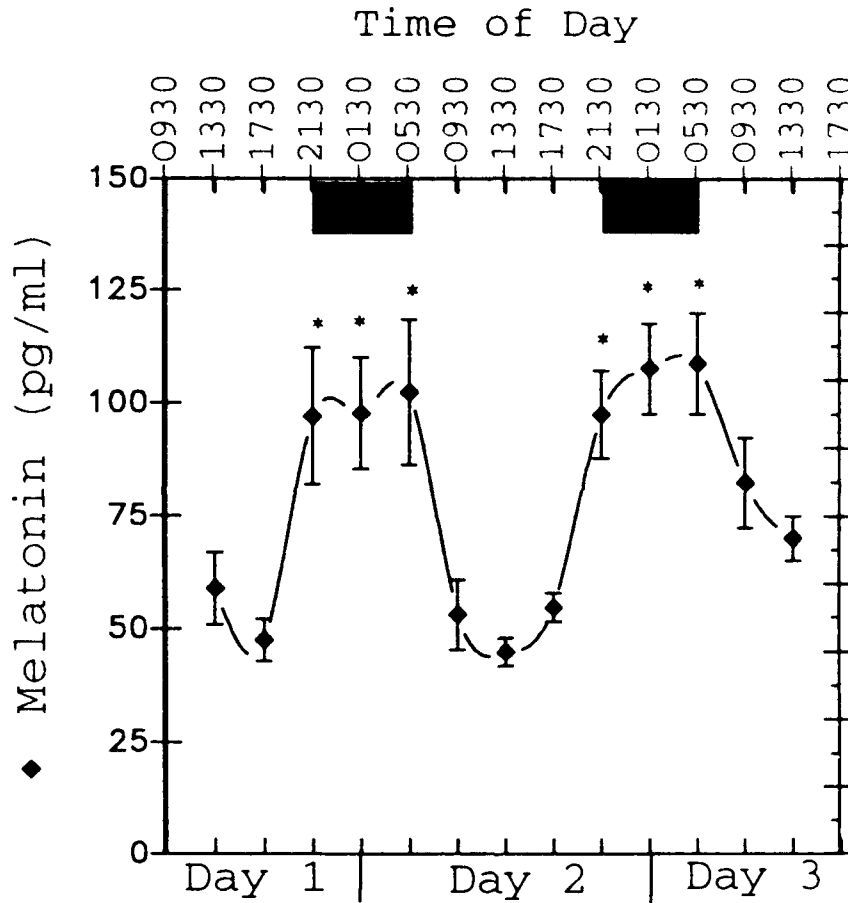


Figure 3. The effect of photoperiod on plasma melatonin concentrations in cats determined at 4-hour intervals. Data are represented as the mean \pm SEM of seven animals assayed in duplicate at each time period.

Discussion

Results of this study showed that blood AChE and plasma BUCHE activity in the cat did not vary significantly in response to the LD cycle. Elsmore (1981) reported no significant differences in blood cholinesterase activity as a function of time of day in rats. Mabood, Newman, and Nimmo, (1978) showed that variations in enzyme activity, detected in human erythrocytes, did not appear to be circadian or semicircadian in nature. Quay et al. (1971) suggested that results obtained from previous studies in rat brain have led to the conclusion that AChE activities are more closely related to factors other than 24 hour or photoperiod rhythmicity. Our finding that blood cholinesterase activity in cats was not linked to photoperiod agree with these reports on other species.

This study also confirmed results from an earlier report (Leyva, Addiego, and Stabenfeldt, 1984) which linked changes in MEL concentrations to the LD cycle. High MEL concentrations were observed in the dark phase of the LD cycle. The rise in plasma MEL concentrations during the dark phase of the LD cycle and the fall of the concentrations during the light indicates the secretion pattern of MEL is related strongly to photoperiod. It is suggested that the secretion pattern of MEL during a 14:10 LD cycle may be used as a marker for circadian rhythmicity when studying the effects of photoperiod on other compounds.

Acknowledgments

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LTC Patrice Cottebrune
French Army Liaison Office
USAAVNC (Building 602)
Fort Rucker, AL 36362-5021

Australian Army Liaison Office
Building 602
Fort Rucker, AL 36362

Dr. Garrison Rapmund
6 Burning Tree Court
Bethesda, MD 20817

Commandant, Royal Air Force
Institute of Aviation Medicine
Farnborough, Hampshire GU14 6SZ UK

Commander
U.S. Army Biomedical Research
and Development Laboratory
ATTN: SGRD-UBZ-I
Fort Detrick, Frederick, MD 21702

Defense Technical Information
Cameron Station, Building 5
Alexandra, VA 22304-6145

Commander, U.S. Army Foreign Science
and Technology Center
AIFRTA (Davis)
220 7th Street, NE
Charlottesville, VA 22901-5396

Director,
Applied Technology Laboratory
USARTL-AVSCOM
ATTN: Library, Building 401
Fort Eustis, VA 23604

Commander, U.S. Air Force
Development Test Center
101 West D Avenue, Suite 117
Eglin Air Force Base, FL 32542-5495

Aviation Medicine Clinic
TMC #22, SAAF
Fort Bragg, NC 28305

Dr. H. Dix Christensen
Bio-Medical Science Building, Room 753
Post Office Box 26901
Oklahoma City, OK 73190

Commander, U.S. Army Missile
Command
Redstone Scientific Information Center
ATTN: AMSMI-RD-CS-R
/ILL Documents
Redstone Arsenal, AL 35898

Director
Army Personnel Research Establishment
Farnborough, Hants GU14 6SZ UK

U.S. Army Research and Technology
Laboratories (AVSCOM)
Propulsion Laboratory MS 302-2
NASA Lewis Research Center
Cleveland, OH 44135

COL John F. Glenn
U.S. Army Medical Research
& Development Command
SGRD-ZC
Fort Detrick, Frederick, MD 21702-5012

Dr. Eugene S. Channing
7985 Schooner Court
Frederick, MD 21701-3273

USAMRDC Liaison at Academy
of Health Sciences
ATTN: HSHA-ZAC-F
Fort Sam Houston, TX 78234

Dr. A. Kornfield, President
Biosearch Company
3016 Revere Road
Drexel Hill, PA 29026

NVEOD
AMSEL-RD-ASID
(Attn: Trang Bui)
Fort Belvoir, VA 22060

CA Av Med
HQ DAAC
Middle Wallop
Stockbridge, Hants S020 8DY UK

Dr. Christine Schlichting
Behavioral Sciences Department
Box 900, NAVUBASE NLON
Groton, CT 06349-5900

Brazilian Army Liaison Office
Building 602
Fort Rucker, AL 36362

COL C. Fred Tyner
U.S. Army Medical Research
& Development Command
SGRD-ZB
Fort Detrick, Frederick, MD 21702-5012

Director
Directorate of Combat Developments
ATZQ-CD
Building 515
Fort Rucker, AL 36362